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EFFECTS OF INGESTION OF LITHIC PARTICLES ON GROWTH OF THE APPLE SNAIL *POMACEA CANALICULATA* (CAENOGASTROPODA, AMPULLARIIDAE)

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ABSTRACT

Lithic particles are a common feature in the digestive tract of freshwater snails. Their role in the digestive processes has been demonstrated in some microphytophagous and detritivorous species, as they enhance growth, assimilation and reproduction. It has been suggested that they could have the same function in *Pomacea canaliculata*, a macrophytophagous apple snail with powerful jaws and radula, a strongly muscular and cuticularized gizzard and high levels of enzymatic activity. Our aims were to investigate the occurrence of lithic elements in the digestive tract of *P. canaliculata* snails from natural populations through the analyses of digestive contents, as well as the effect of size and availability of lithic particles on growth and growth efficiency through laboratory experiments. Lithic particles are very common in the digestive tract of *P. canaliculata* from natural populations and from laboratory aquaria if they are available in the immediate environment. Such particles are not retained or concentrated differentially in the stomach and they are apparently totally lost in less than four weeks if the supply is interrupted. The frequency of plant material and lithic particles increases from mouth to anus indicating that the retention time increases in the same way. Sand and plant material frequently co-occur in the intestine and in the stomach indicating that both are ingested together. Ground marble had negative effects on the growth of *P. canaliculata* probably due to the sharp edges and pointed ends of these particles. The availability of natural lithic particles (sand) had a positive effect on growth and also a synergic interaction with the availability of food. The growth efficiency was 25.2% higher when sand was available than when it was absent. These effects were more marked in juvenile females than in juvenile males. Our results indicate that growth rates may be underestimated under laboratory conditions if lithic particles are not supplied regularly and that their presence should be standardized to allow reliable comparisons between studies. Our results also indicate that the effects of food availability and plant palatability on the growth of *P. canaliculata* may be modulated by the presence of lithic particles and this may in turn affect the outcome of interactions between apple snails, other snails and macrophytes.

Keywords: invader, feeding, digestion, trophic ecology, growth efficiency.

INTRODUCTION

Lithic particles are common in the digestive tracts of freshwater snails and they seem to play a role, in some cases essential, in the digestive processes of these animals (Dillon, 2000; Pyron & Brown, 2015). However, experimental tests of the effect of these particles on the growth, reproduction and assimilation have seldom been performed (e.g., Colton, 1908; Carriker, 1946; Noland & Carriker, 1946; Storey, 1970; Brendelberger, 1997; Thomas,

2001). Most of these studies were carried out on small sized pulmonates that are usually considered as microphytophagous and detritivorous grazer-scrapers thriving on algae, detritus and senescent plant material (Dillon, 2000; Pyron & Brown, 2015). On the other hand, apple snails (Ampullariidae) are large snails mostly with macrophytophagous habits (Cazzaniga & Estebeñet, 1984; Cowie, 2002; Marchese et al., 2014; Hayes et al., 2015) and they possess very powerful jaws and radula with which they shred living macrophyte tissues

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(Andrews, 1965; Moretto & Nahabedian, 1989; Martín & Negrete, 2007). They also possess a strongly muscular and cuticularized gizzard in the stomach (Andrews, 1965) and high levels of enzymatic activity in different parts of their digestive tract (Godoy et al., 2013; Luo et al., 2015). Even though these mechanical and chemical tools seem to be sufficient enough to process the relatively soft tissues of the aquatic plants usually consumed, Andrews (1965) described the presence of "small stones or grit" in the gizzard of *Pomacea canaliculata* (Lamarck, 1822), and suggested that they play a role in the disintegration of vegetal material.

Pomacea canaliculata is one of the apple snails to which most records of negative impacts on aquatic crops and ecosystem functioning have been attributed (Cowie, 2002; Carlsson et al., 2004; Hayes et al., 2012; Horgan et al., 2014). Most of these impacts are caused directly by their ability to consume, grow and reproduce on a variety of aquatic plants, including semi-aquatic vascular plants that are difficult to ingest and digest due to their toughness (e.g., Boland et al., 2008; Wong et al., 2010). In spite of the relevant and negative impacts caused by the herbivory of *P. canaliculata* there are no specific studies about the occurrence of lithic particles in its digestive tract and their effect on the growth of this snail, which has been listed by the IUCN among 100 of the world's worst invaders (Lowe et al., 2000). The main aims of the present study were to investigate the presence of lithic elements in the digestive tract of *P. canaliculata* from natural populations and to determine their significance in its trophic ecology. In particular, relative to trophic ecology, we investigated the effect of the size and availability of lithic particles on growth and growth efficiency.

MATERIALS AND METHODS

Field Sampling: Lithic Particles in Different Parts of the Digestive Tract

At least 20 adult snails with shell length (SL, measured from the apex to the farthest point on the aperture with a Vernier caliper to the nearest 0.1 mm) greater than 30 mm were collected by hand in three *P. canaliculata* populations from Southern Pampas (Buenos Aires province, Argentina): in El Huáscar stream (36°55'50"S, 61°35'48"W), in Guaminí stream (37°20'47"S, 62°25'20"W) and in an earthen

channel connecting Pigüé and Venado streams (37°11'26"S, 62°40'26"W).

The snails were sacrificed immediately in the field by sudden immersion in water at 80°C. In the lab the snails were stored at -18°C until they were processed. The snails were thawed and dissected. The digestive tract was split into three sections (crop, stomach and intestine; Fig. 1) which were analyzed separately in search of remains of plant material and lithic particles: sand (0.0625 to 2 mm) and fine gravel (2 to 4 mm).

Nine binary (presence-absence) variables resulting from the combination of the digestive content categories (S: sand, G: gravel and V: vegetal material) and the different sections of the digestive tract (C: crop, S: stomach, I: intestine) were established to analyze the digestive contents; the variables were named accordingly (e.g.: SC: sand in the crop). A cluster analysis was performed using the Jaccard coefficient and the median linkage method to show the association between the variables.

Origin and Maintenance of Snails for Laboratory Experiments

All the snails used in the experiments were obtained from egg masses deposited in the

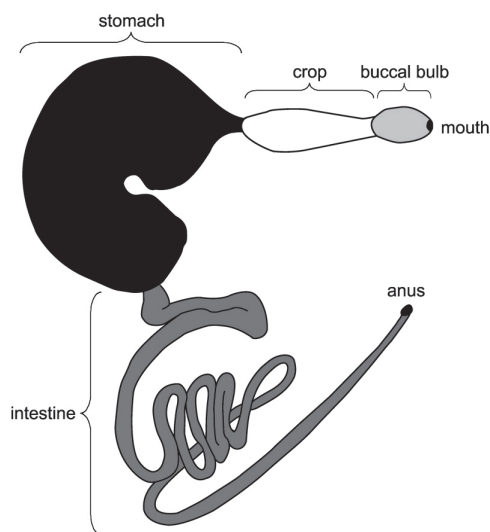


FIG. 1. Scheme of the digestive tract of *P. canaliculata* adults, indicating the different sections of which the contents were analyzed in the present study (crop, stomach and intestine).

laboratory by a stock of snails from El Huáscar stream. The experimental snails were reared since hatching in individual 200 mL plastic aquaria with CaCO_3 -saturated tap water at 25°C, with a 14:10 h (light:dark) photoperiod, and they were fed with lettuce *ad libitum*. The aquaria were cleaned and the water changed once a week. CaCO_3 -saturated tap water was used during the rearing phase to ensure the integrity of the shell. Once the experiments on the snails were started they were transferred to individual 3 L aquaria with non CaCO_3 -saturated tap water to avoid interference of CaCO_3 precipitate with the lithic particles.

Growth Determinations in Laboratory Experiments

All the experiments lasted for four weeks but lithic particles were available since the previous week (week zero; see below). Juvenile snails (between 15 and 20 mm in shell length) were used in all the experiments ($n = 10$ in each treatment, including controls). At the start of each experiment (end of week zero) the initial shell length (SL_0 , mm) of the snails was measured as above and the initial live weight (LW_0 , g) was obtained with a digital scale (± 1.0 mg) after allowing the snails to crawl in a plastic container without water for 30 min to assure the loss of most of the water from the mantle cavity. These measurements were repeated weekly until the fourth week of each experiment.

At the end of the experiments the snails were sacrificed by sudden immersion in boiling water. The snails were stored at -18°C until they were processed. The shell and operculum were removed (except in Experiment 1; see below) and the soft parts were weighed after 72 h at 60°C to obtain their dry weight (SPDW, g). Although the snails were sexually undifferentiated at the start of the experiments, they were dissected before drying and the sex was determined by observation of the penis sheath (males) or the albumen gland (females). The ash content of the soft parts was obtained after 4 h at 600°C and the ash free dry weight (AFDW, g) was calculated.

Experiment 1: Effect of Availability of Lithic Particles

In this experiment ground marble was chosen as a substitute for natural lithic particles due to its mineralogical and granulometric homogene-

ity. Its whitish color also made it easier to detect among the digestive contents and feces, which are usually dark green-brown in this species (Castro-Vazquez et al., 2002). Commercial ground marble was sieved and washed with water before use to obtain particles of similar size (1.285 ± 0.075 mm, mean \pm SE) and free of dust.

We used three treatments to study the effect of the availability of lithic particles on the growth of juvenile snails fed *ad libitum*: continuous (CA), discontinuous (DA) and no availability (NA, control) of lithic particles. In the DA treatment the lithic particles were only available during week zero: 2–2.5 cm³ of ground marble were added after the last water change in the 200 mL aquaria. In the CA treatment the ground marble was provided in the same way during week zero but, after the change to the 3 L aquaria, 2–2.5 cm³ of ground marble were also added every week after the water change.

After the freezing period the soft parts of the snails were removed from the shell and dissected to investigate the presence of ground marble in the stomach and intestine (Fig. 1); the feces at the bottom of the aquaria were also inspected in search of marble particles. Afterwards, the shell and soft parts (including the operculum) were dried for 72 h at 60°C and weighed together to obtain the total dry weight (TDW, g).

Experiment 2: Effect of Size and Availability of Lithic Particles

In this experiment sieved sand obtained from the Napostá stream (38°41'56"S, 62°15'57"W, Buenos Aires province, Argentina) was used. The sand was thoroughly washed in flowing tap water to eliminate the least dense elements (organic debris and pieces of shells) by flotation and drift. The sand was sieved through a series of meshes to obtain two fractions of different diameter (mean \pm SE): 0.80 ± 0.05 and 1.69 ± 0.05 mm (coarse sand and very coarse sand, respectively, according to the Wentworth scale).

Five treatments were designed to test for the effect of size and availability of lithic particles on the growth of juveniles fed *ad libitum*: no availability of lithic particles (NA, control), discontinuous availability of small (DAS) and large lithic particles (DAL) and continuous availability of small (CAS) and large lithic particles (CAL). Lithic particles in the discontinuous availability treatments were only available during week

zero (2–2.5 cm³ of sand were added after the last water change in the 200 mL aquaria), as in Experiment 1, while in the continuous availability treatments the same amount of sand was also added every week after the water change in the 3 L aquaria.

Experiment 3: Effects of Availability of Lithic Particles and Food and their Interaction

In this experiment washed and sieved sand obtained from the Napostá stream was also used. The sand was washed as above and sieved through a series of meshes to obtain a fraction between 1.225 ± 0.05 mm (very coarse sand).

To test for the effects of availability of food and lithic particles on the growth and growth efficiency of juvenile snails, four treatments were performed with a factorial design: food availability (FA) x lithic particles availability (LPA). Two levels of food availability were set: 100% and 50% of the *ad libitum* ingestion of lettuce (FA 100% and FA 50% respectively). The amount of food in week *i* (F_i , provided in two doses) was estimated on the basis of SL_{i-1} (previous week) using the allometric equation developed by Tamburi & Martín (2009b) for *P. canaliculata* daily ingestion rates. Two levels of availability of lithic particles were used: no availability (NA) and continuous availability (CA); in the latter, 2–2.5 cm³ of sand were added after the last water change in the 200 mL aquaria and every week after the water change in the 3 L aquaria.

Growth efficiency in live weight (GELW, g·g⁻¹) was estimated on the basis of the increase in live weight and the accumulated amounts of food provided each week (F_i , g): $GELW = (LW_4 - LW_0) / (F_1 + F_2 + F_3 + F_4)$ throughout the experiment.

Statistical Analysis of Experimental Data

The data from the laboratory experiments were analyzed through one-way ANOVAs (Experiment 1 and Experiment 2) and two-way ANOVAs (Experiment 3). Levene's test was previously performed to investigate homoscedasticity; when homogeneity of variances was rejected for a given variable, the data corresponding to that variable were transformed (see Results). If no transformation was able to homogenize the variances of that variable, the data were analyzed through Kruskal-Wallis tests in the Experiment 1 and in Experiment 2;

in the case of Experiment 3, the factorial design was split by food availability for some variables (see Results) and the difference between snails with and without sand was analyzed by Kruskal-Wallis tests. Fisher's LSD test (least significant difference test) was performed to locate differences between means.

In some experiments the Levene's test and graphical analyses of data suggested that there were differences between the juvenile snails that were already sexually differentiated. In such cases, we split the data and each sex was analyzed separately as above.

RESULTS

Field Sampling: Lithic Particles in Different Parts of the Digestive Tract

The majority of the individuals dissected (93.6%) had lithic particles at least in one section of their digestive tracts. The digestive contents in the three populations were quite similar; the main overall pattern was an increase in the three categories of contents from mouth to anus (Fig. 2). The frequency of lithic particles in the stomach was lower than in the intestine, whereas that of vegetal material was almost the same. Gravel was always the least represented category throughout the digestive tract.

The association in the presence of sand and vegetal material was high both in the intestine and in the stomach and it was also quite similar between these two sections of the

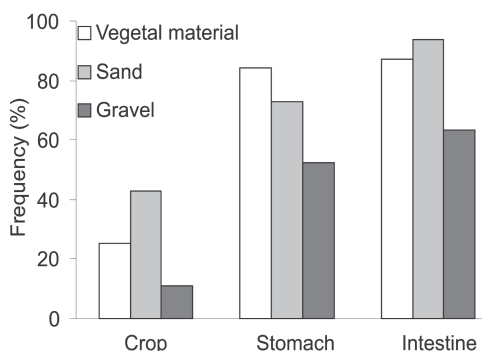


FIG. 2. Frequency of occurrence of vegetal material and lithic particles (sand and gravel) in different sections of the digestive tract for *P. canaliculata* adult snails from the Southern Pampas.

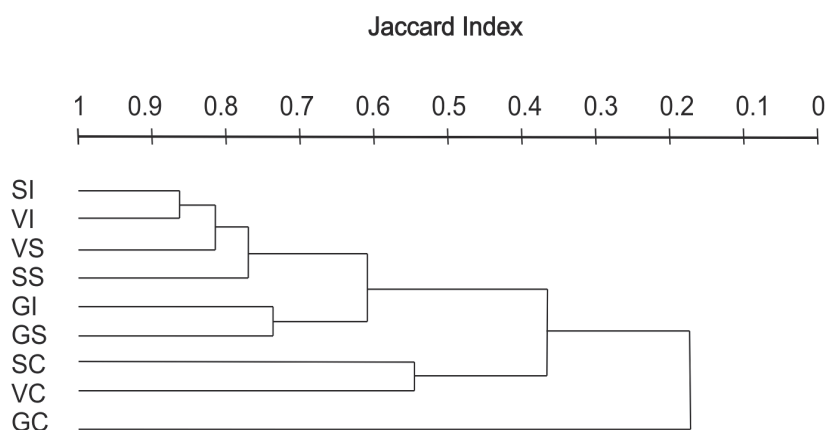


FIG. 3. Dendrogram of association of the contents (S: sand, G: gravel and V: vegetal material) from different sections of the digestive tract (C: crop, S: stomach and I: intestine) for *P. canaliculata* adult snails from the Southern Pampas.

digestive tract (Fig. 3). The presence of gravel in the intestine was highly associated with the presence in the stomach. Sand and vegetal material showed a low degree of association in the crop; gravel in the crop showed the lowest association with other contents in other sections since it was totally absent in the crops of one population and in 95% of the crops of another one.

Experiment 1: Effect of Availability of Lithic Particles

The effect of ground marble on the growth of *Pomacea canaliculata* was mostly negative (Table 1; Figs. 4–6): the snails with continuous availability of ground marble (CA) showed significantly lower final values of SL and TDW than the other two treatments. The final LW of the discontinuous availability treatment (DA) was significantly higher than the control without any marble (NA) and the continuous availability one (CA). When males and females were analyzed separately the negative effects of ground marble on the final values of SL, LW and TDW were not significant (males: SL ($F_{2,19} = 2.967$; $p = 0.076$), LW ($F_{2,19} = 1.811$; $p = 0.191$) and TDW ($F_{2,19} = 2.179$; $p = 0.141$); in the case of females, t-tests for unequal variances were performed as there were no females in the control: SL ($t_{2,255} = 1.429$; $p = 0.276$), LW ($t_{2,195} = 1.655$; $p = 0.229$) and TDW ($t_{2,217} = 1.711$; $p = 0.217$)).

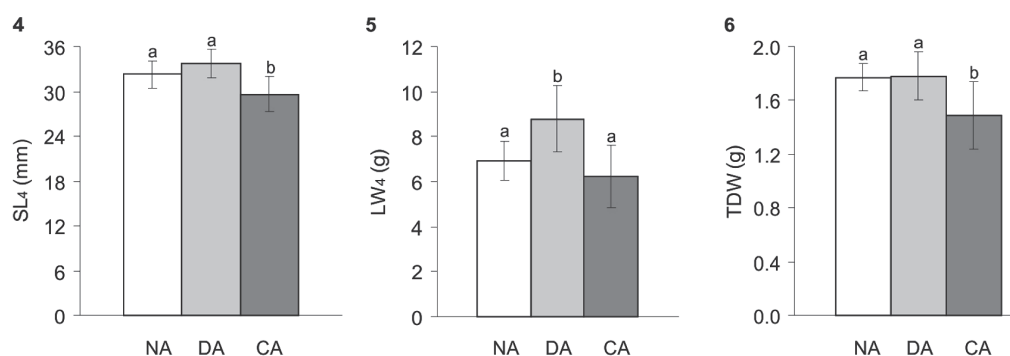
The dissections performed after the Experiment 1 showed that 60% of the CA snails had marble particles in their guts (mostly in the stomach) while none of the DA snails showed them. The feces also showed marble particles included in a matrix of lettuce remains and pigmented corpuscles.

Experiment 2: Effect of Size and Availability of Lithic Particles

No significant effects of size and availability of lithic particles were detected on SL_4 ($F_{4,45} = 0.376$; $p = 0.825$), LW_4 ($F_{4,45} = 0.354$; $p = 0.840$), $SPDW$ ($F_{4,45} = 0.161$; $p = 0.957$) and $AFDW$ ($F_{4,45} = 0.265$; $p = 0.899$). The dissections performed after the experiment showed that 45 out of 50 experimental snails were juvenile males.

TABLE 1. One-way ANOVAs for the effect of availability of lithic particles on growth of juveniles of *P. canaliculata*. Abbreviations: final shell length (SL_4), final live weight (LW_4), total dry weight (TDW).

	$F_{2,27}$	p-value	MS Error
SL_4	5.318	0.011	7.933
LW_4	5.596	0.009	3163461
TDW	3.983	0.030	68797



FIGS. 4–6. The effect of availability of lithic particles on growth of juveniles of *P. canaliculata* (mean \pm 95% CI); no availability (NA), discontinuous availability (DA), continuous availability (CA). FIG. 4: Final shell length (SL₄); FIG. 5: Final live weight (LW₄); FIG. 6: Total dry weight (TDW). Treatments with the same letter were not significantly different ($p > 0.05$) after Fisher's LSD test.

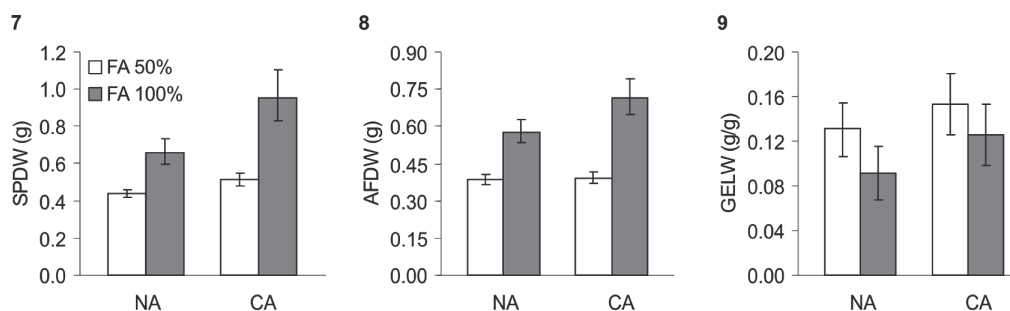
Experiment 3: Effects of Availability of Lithic Particles and Food and their Interaction

The effects of food availability and lithic particles on the final values of SL, LW, SPDW and AFDW were significant and positive (Table 2; Figs. 7–11); the interaction of the two factors was also significant and positive. The SPDW of snails fed *ad libitum* was 44.5% higher when lithic particles were available to them

whereas their effect on snails with reduced food availability was only 17.3% (Fig. 7). The corresponding comparative values for AFDW (Fig. 8), SL₄ (Fig. 10) and LW₄ (Fig. 11) were 23.6 vs. 2.4%, 17.3 vs. 4.8% and 53.8 vs. 15.3%, respectively. The synergistic effect of food and lithic particle availability on the latter two variables was already apparent in their respective growth curves in week one (Figs. 10, 11) but they increased with time until the

TABLE 2. Two-way ANOVAs for the effect of lithic particle (LPA) and food availability (FA) and their interaction (FA \times LPA) on growth and growth efficiency of juveniles of *P. canaliculata*. Abbreviations: final shell length (SL₄), final live weight (LW₄), soft parts dry weight (SPDW), ash free dry weight (AFDW), growth efficiency in LW (GELW). SPDW and AFDW were logarithmically transformed.

		LPA	FA	FA \times LPA	MS Error
SL ₄	F _{1;36}	12.362	14.490	4.246	7.378
	p-value	0.001	0.000	0.047	
LW ₄	F _{1;36}	15.815	22.532	6.182	1631464
	p-value	0.000	0.000	0.018	
SPDW	F _{1;36}	37.037	142.789	5.784	0.004
	p-value	0.000	0.000	0.021	
AFDW	F _{1;36}	12.661	232.055	8.077	0.002
	p-value	0.001	0.000	0.007	
GELW	F _{1;36}	6.038	8.462	0.267	0.001
	p-value	0.019	0.006	0.609	



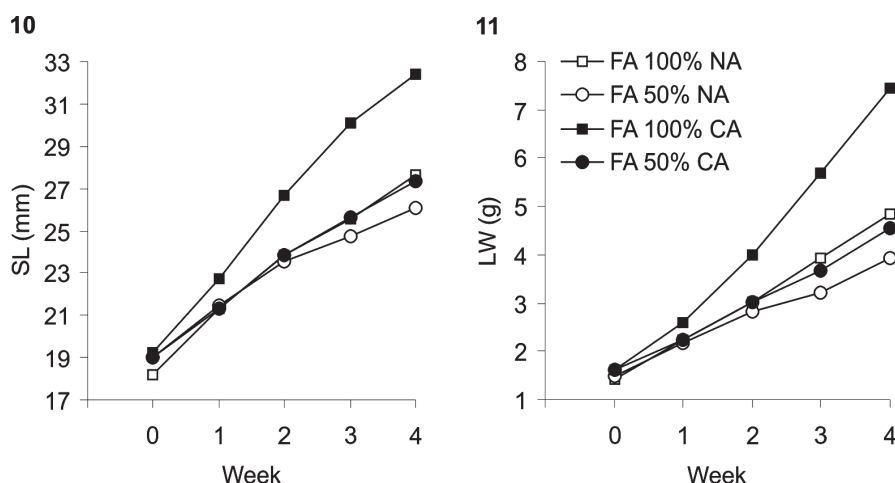
FIGS. 7–9. The effect of availability of lithic particles (NA, no availability and CA, continuous availability) and food (FA 100% and FA 50%) on growth and growth efficiency of juveniles of *P. canaliculata* (mean \pm 95% CI). FIG. 7: Soft parts dry weight (SPDW); FIG. 8: Ash free dry weight (AFDW); FIG. 9: Growth efficiency in LW (GELW).

end of the experiment. The availability of lithic particles allowed the snails with 50% food availability to grow at the same rate as those with 100% food availability but without lithic particles.

On the other hand, the growth efficiency in LW (GELW) showed a significantly positive effect of lithic particles and a significantly negative effect of food availability but there was no significant interaction between these two factors (Table 2; Fig. 9). The GELW of snails fed *ad libitum* and under reduced availability was, on average, 25.2% higher when sand was available than when it was not. The average

GELW of snails fed *ad libitum* was 15.0% lower than the corresponding values of snails with 50% reduction in food availability.

The pattern of the effects of food and lithic particle availability and their interaction on SL_4 , LW_4 , SPDW, AFDW and GELW was the same for juvenile females when they were analyzed separately (Table 3); when lithic particles were available and food availability was low the GELW of females was, on average, 12.0% higher whereas it was 9.0% lower for females fed *ad libitum*. In the case of males, only SL_4 showed a significantly positive effect of food when they were analyzed alone.



FIGS. 10, 11: The effect of availability of lithic particles (NA, no availability and CA, continuous availability) and food (FA 100% and FA 50%) on growth of juveniles of *P. canaliculata*. FIG. 10: Mean shell length (SL); FIG. 11: Mean live weight (LW).

TABLE 3. Two-way ANOVAs for the effect of lithic particle availability (LPA) and food availability (FA) and their interaction (FA x LPA) on growth and growth efficiency of juvenile females and males of *P. canaliculata*: final shell length (SL₄), final live weight (LW₄), soft parts dry weight (SPDW), ash free dry weight (AFDW) and growth efficiency in LW (GELW). SPDW was logarithmically transformed for females and SL₄ was inversely transformed for males. X² values are shown when Kruskal-Wallis tests were performed for the effect of LPA only.

	Variable	Statistic	LPA	FA	FA x PA	MS Error
Females	SL ₄	X ² ₁	4.644			
		p-value	0.031			
	LW ₄	F _{1,22}	39.524	141.725	10.901	283656
		p-value	0.000	0.000	0.003	
	SPDW	F _{1,22}	98.917	373.373	8.079	0.001
		p-value	0.000	0.000	0.009	
	AFDW	F _{1,22}	23.153	241.169	10.741	2120
		p-value	0.000	0.000	0.003	
	GELW	F _{1,22}	9.904	20.042	0.16	0.000
		p-value	0.005	0.000	0.693	
Males	SL ₄	F _{1,10}	2.007	5.533	0.542	0.000
		p-value	0.187	0.040	0.478	
	LW ₄	X ² ₁	0.538			
		p-value	0.463			
	SPDW	X ² ₁	0.360			
		p-value	0.549			
	AFDW	X ² ₁	1.604			
		p-value	0.205			
	GELW	X ² ₁	0.111			
		p-value	0.739			

DISCUSSION

The results obtained in the present study showed that lithic particles are very common in the digestive tract of *Pomacea canaliculata* whenever they are available in the immediate environment. The presence and the characteristics of these lithic particles affect the growth efficiency and the size attained by juvenile snails, especially by the females.

The presence of gravel in all sections of the digestive tract of snails from the natural populations indicates that these particles are not retained or concentrated differentially in the stomach, as Andrews' (1965) observations seemed to suggest. Lithic particles have been

found at high frequencies but with low abundances in the digestive tract of *P. canaliculata* (Kwong et al., 2010; Ocon et al., 2013; López van Oosterom et al., 2013). The frequency of vegetal material, sand and gravel increases from mouth to anus, probably indicating that the retention time in each section also increases in the same way. The crop seems to be the site of short term accumulation of ingested material during bouts of feeding. *Pomacea canaliculata* shows a slightly higher level of activity (Heiler et al., 2008) and of pedal collection of water surface food during the night (Saveanu & Martín, 2013), which may explain why most crops were empty during our diurnal sampling. The intestine is apparently not only

a site of absorption but also of digestion, since proteases are freed to its lumen (Godoy et al., 2013), and this function may be related to an extended retention time relative to other parts of the digestive tract.

The high association of sand and plant material in the intestine and in the stomach probably indicates that the sand was deposited on the plant material and both were ingested together. On the other hand, the presence of gravel in the intestine and the stomach was quite independent of the other two items, probably due to its lower association with plant material. The low degree of association of the crop contents with that of other section is probably due to the high percentage of empty crops. The crop contents only reflect the feeding history of the snail during the few hours prior to collection, whereas those of the stomach, and especially the intestine, integrate the food ingested over several days previous.

The effect of ground marble on growth of *P. canaliculata* was unexpected since the snails with continuous availability attained significantly lower values of shell length and dry weight. In the case of dry weight, the negative effect overcame the additional weight of marble particles in the digestive tract since the dissections showed that the snails with continuous availability still contained these particles in their digestive tracts at the end of the experiment. In the case of final live weight, the effect was inconsistent as under discontinuous availability the snails grew significantly more than in the controls, or than snails under continuous availability. This cannot be explained by the added weight of the marble particles since the dissections showed no particles in their digestive tracts after the four weeks of the experiment.

Accounts of negative effects of lithic particles are rare in the literature. For instance, Schmölder & Becker (1990) found greater shell growth rates and production of eggs and egg reserves during four weeks in snails without sand as compared to those with it although later the differences disappeared despite the active ingestion of sand by *Biomphalaria glabrata* (Say, 1818). The negative effects of ground marble in the case of *P. canaliculata* were probably due to injuries to, or clogging of, the digestive tract because of the irregular and flattened shape and the sharp edges and pointed ends of these particles. The fact that our snails ingested ground marble in spite of its negative effects supports the idea that at least some of the lithic particles are ingested accidentally during feeding. The marble par-

ticles were clearly different from those present in natural waterbodies (mostly rounded and smooth) and found in the digestive contents or used in the other two experiments. The opposite effects of lithic particles described in two studies on *B. glabrata* (Schmölder & Becker, 1990; Thomas, 2001) may be due to differences in mineralogy, size and shape of the particles used in the experiments.

The effect of availability of natural lithic particles (sand) on all the variables related to the somatic growth of *P. canaliculata* (SL₄, LW₄, SPDW and AFDW) was significant and positive. This positive effect has also been described in different species of freshwater pulmonate snails (Colton, 1908; Carriker, 1946; Noland & Carriker, 1946; Storey, 1970; Brendelberger, 1997; Thomas, 2001), which also ingest selectively and retain sand grains. Three main mechanisms have been suggested to explain these positive effects: an increase in the mechanical digestion of food, an increase in the availability of micronutrients and the digestion of microbiota inhabiting the sand grains (Thomas, 2001). The last hypothesis seems implausible in our case since the sand grains were thoroughly washed and then maintained dry until use and were renewed weekly.

The growth efficiency of snails when sand was available was 25.2% higher than when it was absent. This increase in growth efficiency seems too high to be explained only by the effect of micronutrients. Probably the presence of sand allowed a greater breakdown of vegetal tissues and cells, as was suggested by Andrews (1965), and hence a higher efficiency in the extraction of intracellular organic compounds and perhaps a greater relative surface area for the action of digestive enzymes (Godoy et al., 2013; Luo et al., 2015).

The presence of sand had a synergistic effect with food availability on the growth rates of *P. canaliculata* juveniles, especially for females. Under low food availability the digestive efficiency was higher, as indicated by the high values of growth efficiency, but the total effect on growth was low due to the scarce amount of food available. On the other hand, when food availability was high the digestive efficiency was probably lower but the addition of sand allowed a more complete disintegration, which coupled with the abundant vegetal material allowed a multiplicative effect on the growth rates.

In the case of the juvenile males of *P. canaliculata* in the third experiment, the presence of sand had no effect and the food availability

only provoked an increase in shell length. In this case the lack of significant effects may be due to the small number of replicates but this is not the case in the second experiment. In the second experiment no effect of the size of lithic particles or its availability was found, probably due to the fact that 90% of the juveniles were males, an odd result but compatible with the oligogenic sex-determination mechanism in this species (Yusa, 2007). The males of *P. canaliculata* have lower growth rates than females even before maturity is attained (Estoy et al., 2002; Estebenet & Martín, 2003; Tamburi & Martín, 2009a) and so the experimental error in the estimations of male growth would have been proportionally higher. On the other hand, the digestive gland of males is 28–39% smaller than that of females (Vega et al., 2005) due to a greater portion of the visceral mass being occupied by the testicle than by the ovarium, which may have impeded them from taking advantage of the higher disintegration of food.

Pomacea canaliculata females apparently profit more than males from the presence of sand. This species is usually dimorphic in shell size, with females attaining large sizes more frequently than males (Cazzaniga, 1990; Estebenet & Martín, 2002; Tamburi & Martín, 2009a). However, the differences in size between sexes differ notably among populations (Estebenet et al., 2006), probably due to the sexually dimorphic reaction norms to food availability (Tamburi & Martín, 2009a). Perhaps the variation in the availability and composition of particles among sites enhances or diminishes the size dimorphism between sexes due to different effects on the growth rates of both sexes.

Several studies showed that although apple snails are generalist herbivores they feed selectively and grow differently on diverse macrophytes (Estebenet, 1995; Lach et al., 2000; Morrison & Hay, 2011), in part due to the differences in the mechanical defenses of the latter (Boland et al., 2008; Wong et al., 2010). Experiments using reconstituted plants showed that the physical defenses negatively affect the ingestion rates of *P. canaliculata* (Wong et al., 2010). This may be attributed to the difficulties in chewing and shredding the tissues of plants with high dry matter content, although a hindrance to the mechanic digestion along the digestive tract may also be important. Storey (1970) mentioned that *Radix peregra* (Müller, 1774) eats aquatic macrophytes more readily and at a higher rate when sand grains are

present. Probably some plants that appeared to promote little growth, survival and fecundity in *P. canaliculata* (Qiu & Kwong, 2009) would have had more beneficial effects if lithic particles have been available. Brendelberger (1997) found that the addition of sediment increases the growth of two freshwater snails when feeding on certain trophic items (diatoms, green algae and maple leaf particles) but not when feeding on others (blue green algae).

In the third experiment the final sizes attained by the snails fed *ad libitum* were 17.3 to 53.8% higher when sand was available. These results indicate that the growth rates estimated for *P. canaliculata* in most laboratory studies (e.g., Tamburi & Martín, 2009b; Seuffert & Martín, 2013) were in fact underestimations of the maximum growth rates that this snail can reach under *ad libitum* conditions. Other studies used shell oyster powder or coral sand to provide calcium to apple snails (e.g., Estoy et al., 2002; Garr et al., 2011) and probably these materials play the role as lithic particles. However, the sign and magnitude of the effects may depend on the size and shape of the shell or coral fragments used, as our experiment with ground marble showed. These results indicate that the presence of lithic particles should be standardized as well as other details of the rearing conditions, in order to allow meaningful comparisons between studies on apple snail biology.

As in other freshwater snails, the lithic particles have a quantitatively important effect on growth and probably also on reproduction but they cannot be considered as essential (but see Storey, 1970). For instance, in *P. canaliculata* the availability of lithic particles allowed the snails to compensate for reductions in food availability up to 50%, indicating their importance in waterbodies where availability is naturally low or fluctuating or where it has been reduced by the herbivory of the apple snails on the submerged macrophytes (e.g., Carlsson et al., 2004). On the other hand, negative effects of lithic particles appear under certain circumstances (our study; Schmölder & Becker, 1990). The magnitude and sign of species interactions frequently vary according to the abiotic and biotic context (Chamberlain et al., 2014; Fröh et al., in press). Our results indicate that the outcome of interactions among apple snails and other snails, and also with macrophytes, may be modulated by the effects of the sediment traits (size, shape and mineralogy) on the growth and reproduction of the snails.

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